MOLECULAR AND CELLULAR BIOLOGY, Aug. 1987, p. 2947–2955
0270-7306/87/082947-09$02.00/0

Vol. 7, No. 8

Saccharomyces cerevisiae SSB1 Protein and Its Relationship to Nucleolar RNA-Binding Proteins

AMBROSE Y.-S. JONG,† MICHAEL W. CLARK, MARY GILBERT, ALEXANDER OEHM, AND JUDITH L. CAMPBELL*

Divisions of Biology and Chemistry, California Institute of Technology, Pasadena, California 91125

Received 9 February 1987/Accepted 27 April 1987

To better define the function of *Saccharomyces cerevisiae* SSB1, an abundant single-stranded nucleic acid-binding protein, we determined the nucleotide sequence of the *SSB1* gene and compared it with those of other proteins of known function. The amino acid sequence contains 293 amino acid residues and has an M_r of 32,853. There are several stretches of sequence characteristic of other eucaryotic single-stranded nucleic acid-binding proteins. At the amino terminus, residues 39 to 54 are highly homologous to a peptide in calf thymus UP1 and UP2 and a human heterogeneous nuclear ribonucleoprotein. Residues 125 to 162 constitute a fivefold tandem repeat of the sequence RGGFRG, the composition of which suggests a nucleic acid-binding site. Near the C terminus, residues 233 to 245 are homologous to several RNA-binding proteins. Of 18 C-terminal residues, 10 are acidic, a characteristic of the procaryotic single-stranded DNA-binding proteins and eucaryotic DNA- and RNA-binding proteins. In addition, examination of the subcellular distribution of SSB1 by immunofluorescence microscopy indicated that SSB1 is a nuclear protein, predominantly located in the nucleolus. Sequence homologies and the nucleolar localization make it likely that SSB1 functions in RNA metabolism in vivo, although an additional role in DNA metabolism cannot be excluded.

Eucaryotes contain multiple species of proteins that bind preferentially but without sequence specificity to single-stranded DNA (ssDNA). Since the ssDNA-binding proteins (SSBs; the term SSB has traditionally been used to describe proteins that bind ssDNA and whose in vivo role involves binding to DNA, and this definition will be adhered to in this paper) specifically stimulate certain DNA polymerases, it was at first thought that they were involved in unwinding DNA during replication, recombination, and repair, as has been shown for their procaryotic counterparts (for reviews, see references 4 and 18). Still, one protein carries out all these functions in bacteria, and there was no good explanation for the multiplicity of SSBs in eucaryotic systems. It is now clear that an analogy to the procaryotic prototype was much too simple a view and that the physical heterogeneity of eucaryotic SSBs reflects proteins of many diverse physiological functions. In fact, even in their mechanism of DNA polymerase stimulation, the eucaryotic and procaryotic SSBs are very different. Procaryotic SSBs stimulate cognate polymerases by specific protein-protein interactions. In eucaryotes, however, the stimulation apparently occurs because of a unique feature of DNA polymerase α which, unlike procaryotic DNA polymerases and the other eucaryotic polymerases, is inhibited by naked ssDNA. DNA polymerase α activity is not inhibited, however, if the ssDNA is coated with an SSB (5).

By comparison of protein and DNA sequence data, the structural and functional relationships between the various eucaryotic SSBs that have been described over the years are just now coming to be understood. Interestingly, the SSBs described to date may not really be SSBs at all. A few of these so-called SSBs have turned out to be dehydrogenases that fortuitously bind to DNA, presumably by virtue of their nucleotide-binding sites (5). Furthermore, the importance of other SSBs in eucaryotic RNA metabolism has become obvious. Analysis of amino acid sequences of the prototype eucaryotic SSBs, UP1 and UP2 proteins from calf thymus (11–13) and HDP1 protein from mouse myeloma (38), shows that there is extensive sequence homology with heterogeneous nuclear ribonucleoproteins (hnRNPs). Strongly homologous stretches of sequence can be seen between hnRNP A1 and UP1 (19, 26, 28, 38), between hnRNP A2 and HDP1 (19), and between UP2 and yet another, non-A type hnRNP (21). These hnRNPs are core components of the 40S hnRNP complex, which contains nascent mRNA and is thought to be involved in the transport, stabilization, or processing of mRNA (5). Whether the same protein or related proteins are involved in such different processes as replication and RNA processing remains to be demonstrated.

We have shown that *Saccharomyces cerevisiae*, like other eucaryotes, contains multiple species of SSBs (15, 16). To date, we have characterized a 45-kilodalton SSB, designated SSB1, most extensively. SSB1 was isolated on the basis of preferential binding to ssDNA versus double-stranded DNA (dsDNA) and was subsequently shown also to bind to RNA (16). Although SSB1 seems to bind without sequence specificity, it is interesting to note that it copurifies through several affinity steps with the yeast poly(A)-binding protein (31; A. Sachs and A. Jong, unpublished observations). SSB1 stimulates yeast DNA polymerase I on ssDNA templates, which the polymerase by itself copies inefficiently (16). Because of this property, we felt that the protein might be involved in DNA replication or repair. To investigate this, we cloned the gene and carried out gene disruption experiments to see if the gene was essential and present in a single copy. Surprisingly, strains containing the gene disruption grew normally, even though they were shown to contain no immunologically cross-reacting proteins. Furthermore, the gene was not required for sporulation, spore germination, or recombination. DNA repair was not tested. Because the abundance of the protein (20,000 copies per cell) suggests

* Corresponding author.
† Present address: Department of Pediatrics and Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033.

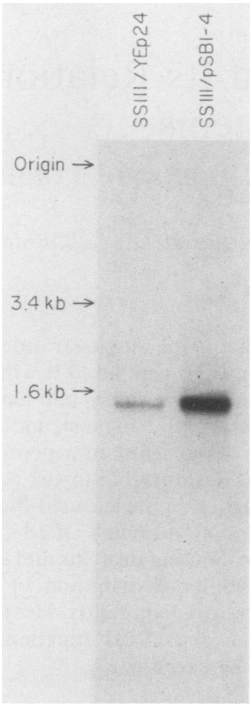

FIG. 1. Northern blot analysis of SSB1 RNA in cells containing the gene on the high-copy-number plasmid pSB1-4. Blot hybridizations were carried out as described in Materials and Methods. A single band of approximately 1.2 kb was detected in each lane. The lane containing RNA from pSB1-4 showed a 10-fold overproduction of wild-type SSB1 mRNA, as determined by scintillation counting of excised bands.

that it has an important biological role and because of the newly appreciated role of many eucaryotic SSBs in RNA metabolism, we sought additional approaches to help elucidate the role of SSB1. The two lines of evidence presented in this paper suggest a role in nuclear RNA metabolism but do not rule out a role in DNA metabolism.

MATERIALS AND METHODS

RNA analysis. Poly(A)$^+$ RNA was isolated from yeast strain SS111 *tyr1 ura3-52 ade2-10* transformed with either YEp24 or pSB1-4 (YEp24 containing the *SSB1* gene insert [14]) by the method of Domdey et al. (7). The RNA was fractionated by electrophoresis on a 1.3% agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose as described by Maniatis et al. (25). A nick-translated *Eco*RI-*Hpa*I fragment of the cloned *SSB1* gene was used to probe the filter. Washes were carried out at 55°C in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.05% Sarkosyl.

Preparation of subclones. The 1.93-kilobase (kb) *Bgl*II fragment previously shown to contain the entire *SSB1* gene, including all essential 5′- and 3′-flanking regions (15), was cloned into the *Bam*HI site of M13mp18 in both orientations and cultivated in *Escherichia coli* JM109 (39). The sense orientation was designated BGL4, and the anti-sense orientation was designated BGL6. A series of deletions was produced by a modification of the method of Henikoff (10). ssDNA was prepared by the method of Hines and Ray (14).

Sequencing. The *SSB1* gene and flanking regions were sequenced by the method of Sanger et al. (32, 33) as modified by Biggin et al. (3). [^{35}S]dATP (500 Ci/mmol) and M13 15-base primer from Bethesda Research Laboratories, Inc., were used. The sequence data were analyzed by using software developed by A. Goldin at the California Institute of Technology.

Homology and structure studies. The protein sequence predicted by the DNA sequence was used to screen the GENBANK Data Base for regions of homology with other DNA- or RNA-binding proteins. The region from residues 125 to 162 containing the repeated sequence RGGFRG was analyzed by using graphics software developed by S. Mayo at the California Institute of Technology to predict its secondary structure.

Localization of the SSB1 gene product in yeast cells. Immunofluorescence microscopy was carried out by a modification of the techniques of Adams and co-workers (2, 17). Wild-type *S. cerevisiae* YM214 was grown to an A_{590} of 0.5 and harvested. Cells were washed and fixed in 3.7% formaldehyde in 0.1 M KPO_4 (pH 6.5) for 90 min. The fixed cells were washed and treated with a mixture of β-glucuronidase (Sigma Chemical Co.) and Zymolyase. The spheroplasts were mounted on polylysine-coated glass slides, extracted with methanol and then with acteone, and allowed to air dry. A 1-mg/ml solution of affinity-purified rabbit anti-SSB1 immunoglobulin G was applied and incubated for 17 h at 6°C. Slides were washed with buffer and incubated with a 1/100 dilution of anti-rabbit immunoglobulin G–fluorescein isothiocyanate conjugate (Sigma). Cells were washed, 1 drop of 90% glycerol containing 1 mg of propyl gallate per ml was applied, and a cover slip was attached. Slides were viewed with a Zeiss standard microscope by using Nomarski interference optics and fluorescein isothiocyanate excitation wavelengths.

RESULTS AND DISCUSSION

Determination of the size of SSB1 mRNA. Samples of poly(A)$^+$ mRNA from wild-type yeast strain SS111 transformed with YEp24 or pSB1-4 were fractionated by size on a denaturing gel by electrophoresis, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled probe of the *Eco*RI-*Hpa*I fragment of the cloned *SSB1* gene (15). The probe detected a single band of about 1.2 kb in length (Fig. 1). The lane containing RNA from SS111(pSB1-4) showed a 10-fold overproduction of the mRNA, as compared with the SS111(YEp24) lane, as determined by quantitation of the radioactivity in the bands after excision from the gel, confirming that the band in lane 1 encoded SSB1. The size of the mRNA was sufficient to encode 45-kilodalton SSB1.

It was somewhat unexpected that only a single 1.2-kb RNA was observed. In contrast, Southern analysis with the same *Eco*RI-*Hpa*I internal fragment as the probe revealed the presence of two bands (15). One corresponded to the authentic *SSB1* gene, and the second, more weakly hybridizing band, suggested that there might be a second, related gene in *S. cerevisiae* (15). Since SSB1 is an abundant protein, it is likely that the mRNA is also relatively abundant (15, 16). If there is a second, related gene, it must either have been the same size as *SSB1*, 1.2 kb not being an unusual length, or it could have been less abundantly transcribed and gone undetected in the experiment shown in Fig. 1.

Sequencing of the *SSB1* gene. To better define the biological roles of SSB1 and its structural relationship to other eucaryotic SSBs, we sequenced *SSB1* DNA. The strategy used for sequencing the 1.93-kb *Bgl*II fragment containing the gene is shown in Fig. 2. Deletions were made with

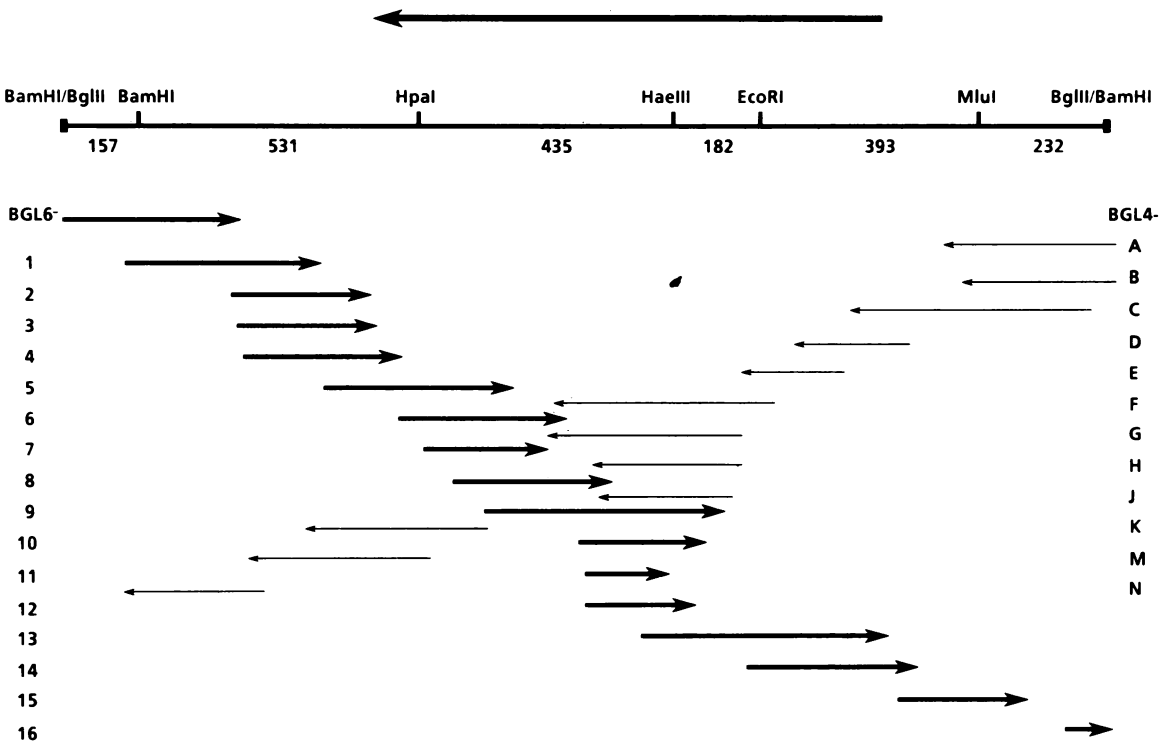

FIG. 2. Sequencing strategy for the *SSB1* gene. A 1.93-kb *Bgl*II fragment containing the gene was cloned into the M13mp18 *Bam*HI site in both orientations and was sequenced as described in Materials and Methods. The boldface arrow above the restriction map shows the location of the open reading frame and the direction of transcription. Below the map the arrows indicate the position, length, and direction of the sequenced fragments (boldface arrows for antisense clones and lightface arrows for sense clones). The sense clones are on the right side of the figure, and the anti-sense clones are on the left side.

exonuclease III by a modification of the method of Henikoff (10). The sequence was determined in both orientations four to seven times by the dideoxy chain termination method. The DNA sequence and translation of the open reading frame are shown in Fig. 3. The accuracy of the sequence was judged in two ways. First, the experimentally determined restriction map was shown to coincide with that derived from the sequence. Second, and more importantly, the deduced open reading frame was confirmed by a comparison with the previously determined amino acid sequences of five CNBr- and trypsin-cleaved peptides of SSB1, peptides P1 to P5 (16). All of the peptides were accounted for in the DNA sequence (Fig. 3). Minor differences can be explained as follows. P3 differed from P5 only in its N terminus, and because the phenylthiohydantoin amino acid sequencing signals were ambiguous (16), we assume that P3 is the same as P5. The N terminus of P1 described in reference 16 also differed from that in Fig. 3. However, this difference can be attributed to the ambiguous phenylthiohydantoin signals obtained for this end of the peptide (16). Since the majority of the P1 peptide amino acids were identical to those in the open reading frame, we presume that these sequences coincide. Also, considering that the DNA sequence was determined multiple times without variation, we conclude that the sequence in Fig. 3 is correct.

Analysis of the sequence shows that a possible initiation signal for transcription 56 bases upstream from the first ATG is the less common TCGA (8). The only TATA sequence in the 5'-flanking region is 397 bases upstream from the start codon and thus is unlikely to serve as an initiation signal. There are 5 in-frame and 13 out-of-frame termination codons between the end of the open reading frame and the start of the poly(A) sequence. A possible poly(A) addition site is 319 bases 3' from the first termination codon. The open reading frame extends from nucleotides 446 to 1327 and codes for a protein of 293 amino acids with a molecular weight of 32,853. This size is much smaller than the 45 kilodaltons suggested by the comigration of ovalbumin and SSB1 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Still, expression of this gene in *E. coli* under T7 promoter control produced a protein with the same antigenic and electrophoretic mobilities as wild-type SSB1 (15), indicating that we have the entire *SSB1* gene. We believe that the ATG indicated is the first methionine in SSB1, since no upstream ATGs in any reading frame are in-frame with the open reading frame shown in Fig. 3. Notably, there are no tryptophan residues and only one cysteine residue in SSB1.

The sequence shows that the *Eco*RI-*Hpa*I fragment used for the original gene disruption interrupts the gene on the C-terminal side, only 27 amino acids away from the N terminus. Since gene disruption was carried out by using this internal fragment containing only a small insertion at the *Hae*III site, we must reevaluate the essentiality of SSB1 in *S. cerevisiae*. If gap repair removed the insertion, the disruption should only have removed 27 amino acids from the protein, an amount which might not impair function. If gap repair did not remove the insertion, there would be a frameshift after the *Hae*III site, resulting in no protein or a defective one. While the latter is more consistent with the protein blots that show no cross-reacting material in the gene-disrupted cells, we do not know which is the case; a different strategy of gene disruption is required to confirm

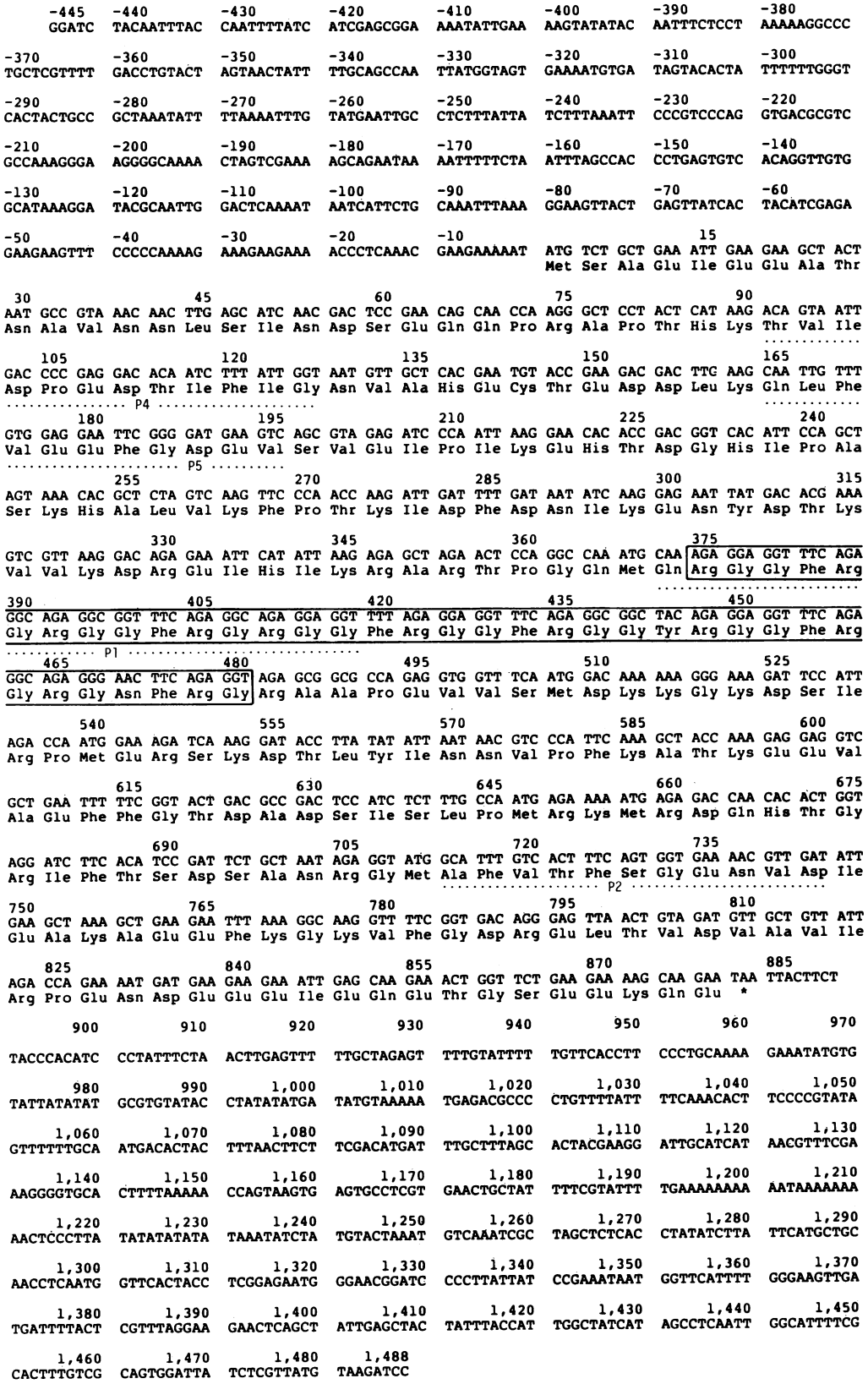

FIG. 3. DNA sequence and translation of the *Bgl*II fragment of *SSB1*. The boxed region contains the repeated sequence described in the text. The residues underlined with dots correspond to peptides described in reference 16. The asterisk indicates the termination codon.

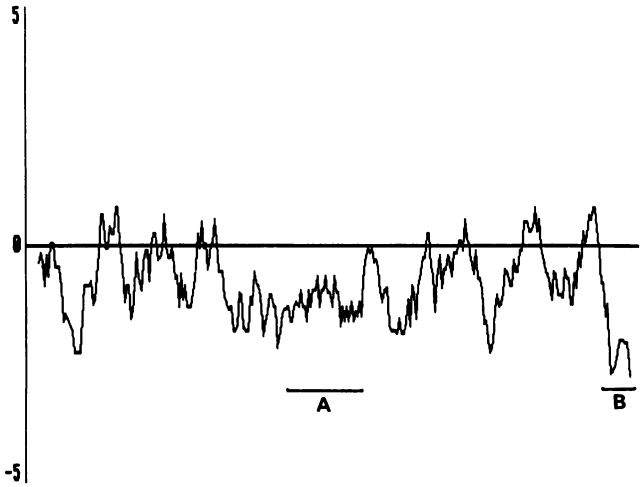

FIG. 4. Hydropathy plot of SSB1 as calcuated by the method of Kyte and Doolittle (20). The window size was 11 residues. Positive values correspond to hydrophobic residues, and negative values correspond to hydrophilic residues. Region A shows the tandem repeat of the sequence RGGFRG. Region B shows the acidic C terminus.

that SSB1 is not an essential protein in *S. cerevisiae*, as previously reported (15).

SSB1 structure. A plot of the hydrophobic character of SSB1 is shown in Fig. 4. The large number of hydrophilic residues and the absence of large regions of highly hydrophobic residues indicate that this protein is probably soluble and not membrane bound (20).

Homology to RNA-binding proteins. The sequence obtained was compared with those of other known RNA- and DNA-binding proteins by computer analysis. There are two regions that are shared between SSB1 and many of these other proteins. The first region is the short peptide IFIGNVAHECTEDDL. It lies near the N terminus of SSB1 (residues 39 to 54) and exhibits 39.3% homology with calf thymus UP1, 35.7% homology with calf thymus UP2, and 35.7% homology with human hnRNP C and other proteins (Table 1, consensus sequence 1). In addition to the identical amino acids, several more are chemically similar ones. Of 15 amino acid residues compared, the amino-proximal side has a neutral amino acid region, and the carboxy-proximal side has an acidic amino acid region.

The second region of homology is also a short peptide, RGMAFVTF, and is located near the C terminus (residues 235 to 241). This sequence is conserved in all RNA-binding proteins that have been sequenced so far (Table 1, consensus sequence 2). Yeast SSB1 has a methionine (residue 237) at a position within the consensus sequence occupied by phenylalanine or tyrosine in all of the other proteins. This methionine is not likely the result of a DNA sequencing error, however, since CNBr, which cleaves specifically at methionine, cleaves SSB1 precisely at residue 237 to produce the peptide labeled P2 in Fig. 3 and reference 16.

The function of these conserved sequences is not known. Conservation between such widely divergent organisms as yeasts and humans is striking, however, and therefore it is reasonable to assume that they represent important structural components of this group of proteins. In addition to domains for DNA binding, single-stranded nucleic acid-binding proteins must also contain specific functional domains, such as nuclear targeting signals and recognition sites for protein-protein interactions, and must have an organization allowing interactions with more than one nucleic acid. The conserved sequence near the N terminus (Table 1, consensus sequence 1) does not have any characteristics known to be important in nucleic acid-protein interactions and may thus be a nuclear targeting signal. The RGMAFVTF motif, on the other hand, contains the aromatic and charged amino acids implicated by physical stud-

TABLE 1. Sequence homology between yeast SSB1 and other nucleic acid-binding proteins

Consensus sequence	Source	Protein	Residue	Sequence[a]	Reference
1	Yeast	SSB1	39	I F I G N V A H E C T E D D L	This study
	Calf thymus	UP1 (hnRNP A1)	15	L F I G G L S F E T T D E S L	26
			106	I F V G G I K E D T E E H H L	26
		UP2		I F V G G L S P D T P E E K	26
	Rat brain	HD2 (hnRNP A1)	78	L F I G G L S P D T T D D S L	6
	Mouse myeloma	HDP1 (hnRNP A2)		L F I G G L S F E T T E E S L	38
	Human hepatoma	hnRNP A2		I F V G G L S P D T P E E K I	21
2	Yeast	SSB1	232	S A N R G M A F V T F	This study
	Calf thymus	UP1 (hnRNP A1)	52	R S R G F G F V T Y	26
			142	G K K R G F A F V T F	26
	Mouse myeloma	HDP1 (hnRNP A2)		G F G F V T F	38
	Human hepatoma	hnRNP A2		N K R R G F C P I T F	21
	Xenopus ovary	hnRNP C	210	H K G F A F V O F	B. W. Wold and F. Preugschat, submitted for publication
	Rat brain	HD2 (hnRNP A1)	52	K R S R G F G F V T Y	6
	CHO cells	Nucleolin	85	G S S K G F G P V T F	22
	Yeast	Poly(A)-binding protein	75	K T S L G Y A Y V N F	31
			163	G K S K G F G F V H F	31
			255	G K L K G F G F V N Y	31
			357	G K S K G F G F V C F	31

[a] Boldface type indicates 100% conservation. Blank spaces indicate that the sequences were aligned by the omission of amino acids in the sequences.

ies of procaryotic SSBs in DNA binding. Merrill et al. (26) have proposed that the composition of the RGMAFVTF motif suggests a DNA-binding function for UP1 (hnRNP A1). UP1 (hnRNP A1) contains two independent globular DNA-binding domains, each of which contains this peptide. A similar hypothesis has been advanced for the poly(A)-binding protein by Adam et al. (1).

The homologies suggest that these proteins may be derived from a common ancestor or may constitute a gene family or both. In fact, calf thymus UP1 appears to be derived from hnRNP A1 by proteolysis (6, 19, 28). This apparent precursor-product relationship between these proteins could be the result of the following. (i) Proteolysis of hnRNP A1 during purification removes the C-terminal domain. The isolated protein is then a degraded artifact. (ii) Proteolysis of one functional protein into another truncated functional protein is a part of the protein maturation process. This process would be analogous to the release of the C terminus of the epidermal growth factor receptor, resulting in the production of an active protein-tyrosine kinase (9). (iii) Nucleic acid-binding proteins are derived from a common ancestor; they are so closely related that we cannot distinguish their differences simply by evaluation of their sequences. The last is the least likely. In any case, yeast SSB1 exhibits homology with short stretches of peptides within various kinds of nucleic acid-binding proteins, suggesting that its role in vivo involves interaction with either RNA or DNA.

A third feature conserved in single-stranded nucleic acid-binding proteins in both procaryotes and eucaryotes is an acidic C terminus, and this is also a characteristic of SSB1. From residues 276 to the terminal residue 293, nine residues are glutamic acid and one is aspartic acid. The acidic C terminus has been shown to reduce the affinity of procaryotic SSBs for DNA and is thought to modulate binding in eucaryotic proteins as well.

Repeated sequence element. As shown in the boxed area in Fig. 3, the central region of SSB1 contains a tandem repeat of the sequence RGGFRG (residues 125 to 161) which appears as three larger homologous peaks and two smaller ones on the hydropathy plot shown in Fig. 4. This pattern is typical of a helical secondary structure, and an alpha helix with this sequence was shown by computer modeling to have the appropriate dimensions for interaction with A-form ssDNA (S. Mayo and J. Campbell, unpublished observations). In addition to this feature, the most notable aspect of this sequence is a high concentration of basic and aromatic amino acids. The contribution of aromatic and positively charged amino acids to the binding of ssDNA by fd gene 5 protein (27), bacteriophage T4 gene 32 protein (29), and *E. coli* SSB (37) has been analyzed by several methods. The studies suggest that the positively charged amino acids interact with the negatively charged phosphodiester backbone of ssDNA while the aromatic amino acids stack with the bases. It has been proposed that this stacking interaction accounts for the ability of SSBs to specifically recognize single-stranded as opposed to double-stranded nucleic acids, since less energy is required to unstack ssDNA than dsDNA. On the basis of these studies, it is reasonable to suggest that the tandem repeat sequence in SSB1 is involved in its binding to single-stranded nucleic acid.

An examination of other RNA- and DNA-binding proteins for sequences with compositions similar to that of the RGGFRG repeat revealed several interesting conserved features (6, 22–24, 30, 34). Several proteins show conservation of the actual primary sequence, RGGFRG. Among them, the Epstein-Barr virus nuclear antigen polypeptide shows the highest degree of sequence homology. This protein binds to chromatin and to the DNA at the origin of DNA replication of Epstein-Barr virus. Thus, it has been suggested that Epstein-Barr virus nuclear antigen may play some role in viral DNA replication (30). It has previously been proposed that proteins such as RNA polymerase that interact with both ssDNA and dsDNA are similar to proteins that bind preferentially to ssDNA. Proteins involved in the initiation of replication might also bind to both ssDNA and dsDNA.

While the hnRNPs do not carry an identical repeat, the 125 C-terminal amino acids of hnRNP A1 form a separate domain from the rest of the protein that is rich in Arg, Gly, and Phe. Removal of this domain by cleavage with trypsin alters the binding properties of the protein. hnRNP A1 binds RNA with greater affinity than ssDNA, while the truncated protein prefers ssDNA and has helix-destabilizing activity. In contrast to the helical structure proposed for the repeat in SSB1, however, the separate domain in hnRNP A1 (HD2) appears to be disordered (6). Another protein that carries a C-terminal repeat composed of Arg, Gly, and Phe is the human nucleolar protein C23, or nucleolin. This Arg-Gly-Phe region is even more similar to the repeat in SSB1 than is the Gly-rich region of hnRNP A1, although the nucleolin repeat still has a primary sequence and length different from those of SSB1 (22, 23, 34). A second, 34-kilodalton nucleolar protein, a scleroderma antigen, also contains Gly-Arg clusters interspersed with Phe (24). The difference in actual sequence of the three common amino acids may give rise to different protein-nucleic acid structures and hence specific functions. These sequence comparisons suggest, however, that SSB1 may be more closely related to nucleolar proteins than to mRNA hnRNPs. Both hnRNPs and nucleolar proteins contain large fractions of Arg residues in the modified form of *N*,*N*-dimethlylarginine (22, 24, 34). We have no evidence as to whether the Arg residues in the repeated portion of SSB1 are dimethlyated. This modification would perhaps serve to stabilize the positive charge against environmental changes, suggesting that this substitution may be important for function.

Localization of SSB1 to the nuclear periphery by immunofluorescence microscopy. Indirect immunofluorescence microscopy with affinity-purified antibody to SSB1 demonstrated that SSB1 is exclusively in the yeast cell nucleus (Fig. 5). Two distinct images of nuclear staining were seen with anti-SSB1 antibody. The predominant image, seen in 70% of the cells, was a crescentlike pattern, filling about one-third of the nuclear periphery. In the remaining cells, a halolike fluorescence that circled the entire nuclear circumference was observed. Neither of the two images of nuclear staining was observed if the anti-SSB1 antibody was incubated with 5 to 10 molar equivalents of purified SSB1 before use (data not shown). Thus, both of the observed fluorescence staining patterns are representative of the nuclear location of SSB1.

We interpret the two fluorescent images seen for SSB1 as two different views of a single staining pattern. This pattern would be expected if SSB1 were found in a caplike structure oriented perpendicular to the long axis of the nucleus. Such a structure would produce the crescentlike and halolike images in about the proportions observed in the anti-SSB1 staining when different cells were viewed from random angles.

It is tempting to speculate that the stained region corresponds to the nucleolus. The yeast nucleolus has also been

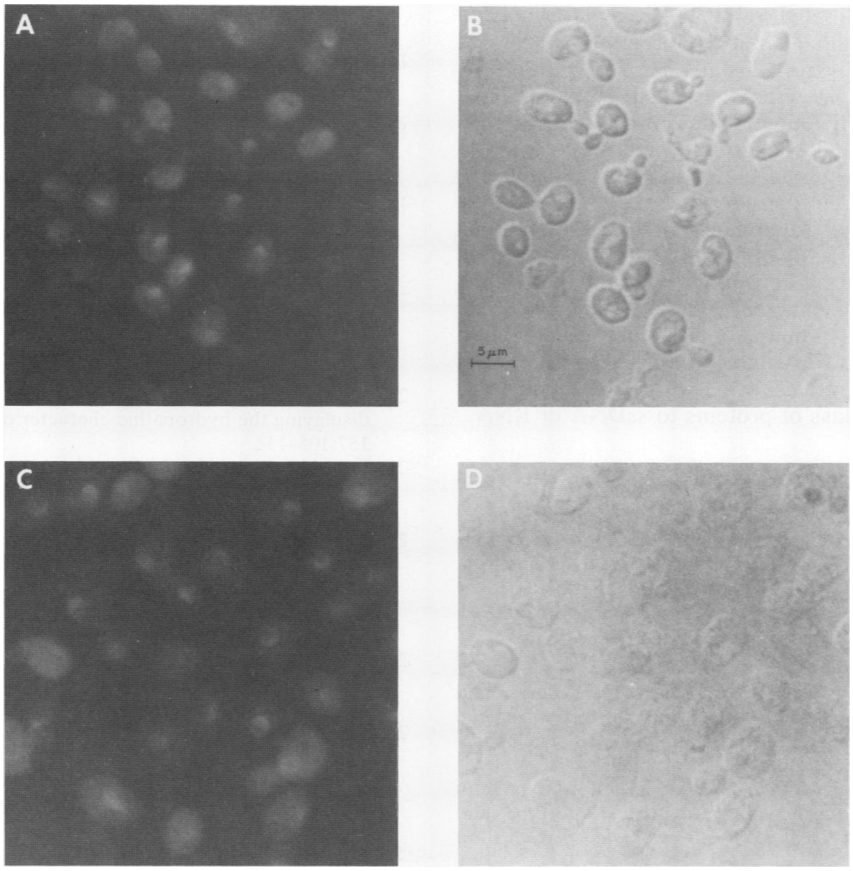

FIG. 5. Subcellular localization of SSB1. (A and C) Immunofluorescent micrographs prepared with anti-SSB1 antibody as described in Materials and Methods. (B and D) Same cells viewed by Nomarski interference optics. The darkest areas correspond to the nuclei, which are often in close apposition to lighter and larger vacuoles.

described as a caplike structure, referred to as the dense crescent, taking up about one-third of the nuclear area (35). To investigate whether SSB1 is localized in the nucleolus, we adapted a nucleolus-specific silver staining method used extensively in studies of the mammalian system to the yeast system (36). The silver stain formed the same proportions of crescent-like and halolike images as were seen with indirect immunofluorescence (Fig. 6). The similarity in the pattern observed with silver and with anti-SSB1 antibody strongly suggests that SSB1 is located predominantly in the nucleolus. This conclusion is also in line with the similarity of the RGGFRG repeat of SSB1 to the C-terminal repeats in nucleolin, a major human nucleolar protein (see above).

Clearly, further studies will be required to completely define the biological roles played by SSB1 and its structural relationship to other eucaryotic single-stranded nucleic acid-binding proteins. However, the studies presented here strongly suggest that SSB1 is a nucleolar protein and show that SSB1 shares many features with proteins whose cellular functions are known to involve RNA binding. Thus, at this point, we favor a role for SSB1 in RNA metabolism rather than DNA metabolism, as had been assumed in the past. The

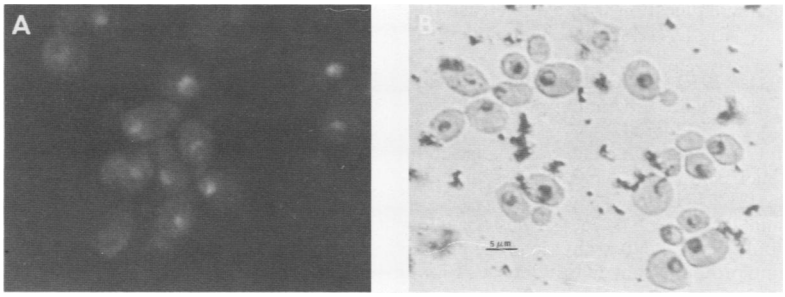

FIG. 6. Comparison of anti-SSB1 staining with nucleolus-specific silver staining. (A) Yeast cells stained wit affinity-purified rabbit anti-SSB1 immunoglobulin G—anti-rabbit immunoglobulin G–fluorescein isothiocyanate conjugate viewed under fluorescein isothiocyanate excitation wavelengths (2, 17). (B) Yeast cells stained with the nucleolus-specific silver stain viewed under bright-field illumination (36).

recognition that SSB1, like UP1 and HDP1, is most likely an RNA-binding protein immediately redirects strategies to identify true DNA-binding proteins in *S. cerevisiae*. It is now clear that one should begin with the yeast strain that we have prepared that carries a deletion of the *SSB1* gene (15), thus substantially reducing the background of unwanted RNA- and DNA-binding activities in the cell extracts. Failure to identify the SSBs required for eucaryotic replication to date may stem solely from the abundance of the RNA-binding proteins. Another important outcome of this work is the discovery of a nucleolar marker for *S. cerevisiae* that will be very useful for cytological and cell biological investigations. Finally, the use of this protein for structure and function studies, especially those involving the internal RGGFRG repeat, should be generally informative as to the mode of binding of this whole class of proteins to ssDNA or RNA.

ACKNOWLEDGMENTS

We thank B. Wold and J. Abelson for stimulating discussions.

This work was supported by Public Health Service grant GM25588 from the National Institutes of Health and by grants from the American Cancer Society and the March of Dimes.

LITERATURE CITED

1. **Adam, S. A., T. Nakagawa, M. S. Swanson, T. K. Woodruff, and G. Dreyfuss.** 1986. mRNA polyadenylate-binding proteins: gene isolation and sequencing and identification of a ribonucleoprotein consensus sequence. Mol. Cell. Biol. **6**:2932–2943.
2. **Adams, A. E. M., and J. R. Pringle.** 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98**:934–945.
3. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
4. **Challberg, M. D., and T. J. Kelly.** 1982. Eukaryotic DNA replication: viral and plasmid model systems. Annu. Rev. Biochem. **51**:901–934.
5. **Chase, J. W., and K. R. Williams.** 1986. Single-stranded DNA binding proteins required for DNA replication. Annu. Rev. Biochem. **55**:103–136.
6. **Cobianchi, F., D. N. SenGupta, B. Z. Zmudzka, and S. H. Wilson.** 1986. Structure of rodent helix-destabilizing protein revealed by cDNA cloning. J. Biol. Chem. **261**:3536–3543.
7. **Domdey, H., B. Apostol, R. J. Lin, A. Newman, E. Brody, and J. Abelson.** 1984. Lariat structures are *in vivo* intermediates in yeast pre-mRNA splicing. Cell **39**:611–621.
8. **Hahn, S., E. T. Hoar, and L. Guarente.** 1985. Each of three "TATA elements" specifies a subset of the transcription initiation sites at the *CYC-1* promoter of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:8562–8566.
9. **Hayman, M. J.** 1986. *erb*-B: growth factor receptor turned oncogene. Trends Genet. **2**:260–262.
10. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**: 351–359.
11. **Herrick, G., and B. Alberts.** 1976. Purification and physical characterization of nucleic acid helix-unwinding proteins from calf thymus. J. Biol. Chem. **251**:2124–2132.
12. **Herrick, G., and B. Alberts.** 1976. Nucleic acid helix-coil transitions mediated by helix-unwinding proteins from calf thymus. J. Biol. Chem. **251**:2133–2141.
13. **Herrick, G., H. Delius, and B. Alberts.** 1976. Single-stranded DNA structure and DNA polymerase activity in the presence of nucleic acid helix-unwinding proteins from calf thymus. J. Biol. Chem. **251**:2142–2146.
14. **Hines, J. C., and D. S. Ray.** 1980. Construction and characterization of new coliphage M13 cloning vectors. Gene **11**:207–218.
15. **Jong, A. Y., and J. L. Campbell.** 1986. Isolation of the gene encoding yeast single-stranded nucleic acid binding protein 1. Proc. Natl. Acad. Sci. USA **83**:877–881.
16. **Jong, A. Y., R. Aebersold, and J. L. Campbell.** 1985. Multiple species of single-stranded nucleic acid-binding proteins in *Saccharomyces cerevisiae*. J. Biol. Chem. **260**:16367–16374.
17. **Kilmartin, J. V., and A. E. M. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. **98**:922–933.
18. **Kowalczykowski, S. T., D. G. Bear, and P. H. von Hippel.** 1981. Single-stranded DNA binding proteins, p. 373–444. *In* P. Boyer (ed.), The enzymes, vol. XIV. Academic Press, Inc., New York.
19. **Kumar, A., K. R. Williams, and W. Szer.** 1986. Purification and domain structure of core hnRNP proteins A1 and A2 and their relationship to single-stranded DNA-binding proteins. J. Biol. Chem. **261**:11266–11273.
20. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
21. **Lahiri, D., and J. O. Thomas.** 1986. A cDNA clone of the hnRNP C protein and its homology with the single-stranded DNA binding protein UP2. Nucleic Acids Res. **14**:4077–4094.
22. **Lapeyre, B., F. Amalric, S. H. Ghaffari, S. V. V. Rao, T. S. Dumbar, and M. J. Olson.** 1986. Protein and cDNA sequence of a glycine-rich, dimethylarginine-containing region located near the carboxyl-terminal end of nucleolin (C23 and 100 kDa). J. Biol. Chem. **261**:9167–9173.
23. **Lischwe, M. A., R. G. Cook, S. A. Young, L. C. Yeoman, and H. Busch.** 1985. Clustering of glycine and N^G,N^G-dimethylarginine in nucleolar protein C23. Biochemistry **24**:6025–6028.
24. **Lischwe, M. A., R. L. Ochs, R. Reddy, R. G. Cook, L. C. Yeoman, E. M. Tan, M. Reichlin, and H. Busch.** 1985. Purification and partial characterization of a nucleolar scleroderma antigen (M_r = 34,000; pI, 8.5) rich in N^G,N^G-dimethylarginine. J. Biol. Chem. **260**:14304–14310.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 384–385. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Merrill, B. M., M. B. LoPresti, K. L. Stone, and K. R. Williams.** 1986. High pressure liquid chromatography purification of UP1 and UP2, two related single-stranded nucleic acid-binding proteins from calf thymus. J. Biol. Chem. **261**:878–883.
27. **O'Conner, T. P., and J. E. Coleman.** 1983. Proton nuclear magnetic resonance (500 MHz) of mono-, di-, tri-, and tetradeoxynucleotide complexes of gene 5 protein. Biochmistry **22**:3375–3381.
28. **Pandolfo, M., O. Valentini, G. Biamonti, and S. Riva.** 1985. Single stranded DNA binding proteins derive from hnRNP proteins by proteolysis in mammalian cells. Nucleic Acids Res. **13**:6577–6590.
29. **Prigodich, R. V., J. Cases-Finet, K. R. Williams, W. Konigsberg, and J. E. Coleman.** 1984. ^1H NMR (500 MH_z) of gene 32 protein-oligonucleotide complexes. Biochemistry **23**:522–529.
30. **Rawlins, D. R., G. Milman, S. D. Hayward, and G. S. Hayward.** 1985. Sequence-specific DNA binding of the Epstein-Barr virus nuclear antigen (EBNA-1) to clustered sites in the plasmid maintenance region. Cell **42**:859–868.
31. **Sachs, A. B., M. W. Bond, and R. D. Kornberg.** 1986. A single gene from yeast for both nuclear and cytoplasmic polyadenylate-binding proteins: domain structure and expression. Cell **45**: 827–835.
32. **Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill, and G. B. Peterson.** 1982. Nucleotide sequence of bacteriophage λ DNA. J. Mol. Biol. **162**:729–773.
33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
34. **Sapp, M., R. Knippers, and A. Richter.** 1986. DNA binding properties of a 110 kDa nucleolar protein. Nucleic Acids Res. **14**:6803–6820.
35. **Sillevis-Smitt, W. W., J. M. Vlak, I. Molenaar, and T. H. Rozijn.** 1973. Nucleolar function of the dense crescent in the yeast nucleus. Exp. Cell Res. **80**:313–321.

36. **Thiebaut, F., J. P. Rigaut, and A. Reith.** 1984. Improvement in the specificity of the silver staining technique for Ag-NOR-associated acidic proteins in paraffin sections. Stain Technol. **59**:181–185.
37. **Williams, K. R., E. K. Spicer, M. B. LoPresti, R. A. Guggenheimer, and J. W. Chase.** 1983. Limited proteolysis studies on the *Escherichia coli* single-stranded DNA binding protein. J. Biol. Chem. **258**:3346–3355.
38. **Williams, K. R., K. L. Stone, M. B. LoPresti, G. M. Merrill, and S. R. Planck.** 1985. Amino acid sequence of the UP1 calf thymus helix-destabilizing protein and its homology to an analogous protein from mouse myeloma. Proc. Natl. Acad. Sci. USA **82**:5666–5670.
39. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.